CODING FORM FOR SRC INDEXING

Microfiche No.		
OTS0538162		
New Doc I.D.	Old Doc I.D.	
86-930000179		
Date Produced	Date Recieved	TSCA section
2/28/93	3/23/93	8D
Submitting Organization		
INTL ISOCYANATE INST		
Contractor		
Document Title		
SPECIFICITY AND GROSS REACTIVITY OF ANTIBODIES TO TDI ISOMERS WITH COVER LETTER DATED 032393		
Chemical Category		
2,4-TOLUENEDIISOCYANATE (584-84-9)		

FINAL REPORT
AM-AB-87

SPECIFICITY AND CROSS REACTIVITY OF ANTIBODIES TO TDI ISOMERS

(Study Period: October 1, 1989-September 30, 1990)

February 28, 1993

INTRODUCTION

An association of toluene [illegible]yanate (TDI) with respiratory sensitization has been recognized for many years. Yet, the etiology of the disease remains uncertain. It was hypothesized that part of the difficulty in determining an immunologic response to TDI might be the result of the routine use of the 80:20 mixture of 2,4:2,6 TDI isomers for antigen preparation. These antigens are in turn used to detect antibody in sensitized individuals in support of an immunologic basis for sensitization.

A study was designed to test the ability of each isomer to detect antibodies produced to the opposing isomer. A guinea pig model was used to prepare antibodies to the 2,4 and 2,6 isomers. The antigens were then assessed for their abilities to detect antibodies to each isomer. It was anticipated that results of this study would provide the basis for recommendation of appropriate antigens for use as diagnostic aids to detect TDI antibodies.

METHODS

Production of antibodies. Two guinea pigs were injected intradermally with 100 ul of 2,4 TDI; two were injected with 2,6 TDI, and two were injected with TD80 (an 80:20 mixture of 2,4:2,6 isomers). Sera were collected 21 days following each immunization.

Antibody assays. The antibody titer and specificity of each antiserum was evaluated by ELISA and ELISA inhibition assays. Plates were coated with 5 ug/ml isocyanate-GSA conjugates. Reagents included alkaline phosphatase conjugated rabbit anti-guinea pig IgG (H&L).

RESULTS

The titers of each of the antisera are shown in Table 2. In all cases, titers were highest with the homologous antigen (the conjugate which contained the TDI isomer used for immunization). Immunization with TD80 provided the most potent antisera. All sera showed cross-reactions between 2,4 and 2,6 isomers.

The specificity of the antibodies was examined with results shown in Figure 1. The best inhibitor of reaction of anti-2,6 TDI antisera was 2,6 TDI-GSA (panel A), and analogously, 2,4 TDI-GSA was the best inhibitor of reactions of anti-2,4 TDI antisera (panel B). Sera from animals immunized with TD80 (panel C) were inhibited best by the 80:20 TDI antigen, followed by the 2,4 TDI antigen, and least effectively by the 2,6 antigen. GSA did not inhibit any of the reactions. On the basis of these analyses, the amount of each isomer conjugate required to effect 50% inhibition of the antigen-antibody reaction was calculated and is listed in Table 3.

Cross-recognition of isomers by the various antibodies was investigated. ELISA plates were coated with either 2,4 TDI-GSA or 2,6 TDI-GSA . Antiserum to 2,4 TDI was absorbed with 2,6 TDI-GSA to remove all reacting antibody, and similarly, antibody to 2,6 TDI was absorbed with 2,4 TDI-GSA. The resulting antisera were subsequently tested to see if they retained antibody activity to the immunizing TDI isomer. The results (Table 4) indicated that some antibody reactivity toward the homologous isomer remained following complete removal of activity toward the opposing isomer. Thus, isomer-specific, as well as cross-reactive antibodies had been produced.

CONCLUSIONS

The results of this study showed that 2,4 and 2,6 TDI were each immunogenic in guinea pigs. When used as an immunogen, in a ratio of 4:1 (as in TD80), the resulting antibodies had four times more reactivity with 2,4 as compared with 2,6 TDI.

Antibodies showed cross-reactivity, and therefore recognition of the opposing isomer. However, in all cases, antibodies reacted best with the isomer used for immunization.

These results emphasize the configurational specificity of immunological reactions and the importance of using well-defined antigens for detection of TDI-specific antibodies. The results in guinea pigs suggest that a mixture of 2,4 TDI and 2,6 TDI antigens should be employed to test for the presence in human sera of antibodies to TDI.

Table 1

Organ Weights from Animals Exposed to MDI Aerosol

Group	Body Wght (g)	Thymus (g)	Cervical Lymph Nodes	Mesenteric Lymph Nodes	Spleen (g)	Lung (g)
MDI Exposure - Day 5 Sacrifice						
77	389	0.64	0.17	0.12	0.68	3.90
81	358	0.66	0.16	0.14	0.66	3.32
84	374	0.55	0.18	0.20	0.62	3.70
85	350	0.51	0.18	0.13	0.61	3.49
86	318	0.60	0.18	0.18	0.67	3.14
X	358	0.592	0.174	0.154	0.648	3.51
MDI Exposure - Day 21 Sacrifice						
78	403	0.60	0.22	0.21	0.73	3.12
79	457	0.42	0.24	0.23	0.62	3.01
80	445	0.47	0.21	0.11	0.49	2.62
82	428	0.49	0.18	0.12	0.46	2.80
83	367	0.37	0.12	0.09	0.42	2.53
X	420	0.47	0.194	0.152	0.544	2.82
Sham Exposure - Day 5 Sacrifice						
67	356	0.42	0.11	0.07	0.66	2.50
68	339	0.66	0.12	0.08	0.79	2.89
69	331	0.43	0.07	0.12	0.50	2.44
70	343	0.55	0.13	0.08	0.61	2.43
71	326	0.37	0.11	0.08	0.58	2.46
X	339	0.486	0.108	0.086	0.628	2.544
Sham Exposure - Day 21 Sacrifice						
72	498	0.54	0.18	0.14	0.68	2.87
73	398	0.46	0.23	0.09	0.52	2.62
74	410	0.40	0.23	0.08	0.56	2.54
75	467	0.55	0.17	0.11	0.64	2.86
76	458	0.47	0.14	0.14	0.74	2.78
X	446	0.484	0.19	0.112	0.63	2.734

Table 2

Titers of Antisera Produced to TDI Isomers

Immunization Isomer	Immobilized Test Antigen			
	2,4 TDI-GSA	2,6 TDI-GSA	TDI_{80}-GSA	GSA
2,4 TDI				
#767	10,240	2,560	2,560	<40
#770	2,560	1,280	1,280	<40
2,6 TDI				
#730	5,120	10,240	2,560	<40
#732	5,120	10,240	2,560	<40
TD_{80}				
#9	5,120	5,120	20,480	320
#10	5,120	5,120	40,960	640

Antibody titers were determined by separate ELISA assays using micro titer plates coated with 2,4 TDI-GSA, 2,6 TDI-GSA, TD_{80}-GSA, or GSA at 5 µg/ml. Guinea pig antibodies were detected using alkaline phosphatase conjugated rabbit anti-guinea pig IgG at 1:800 dilution.

Table 3

Specificity of Antibodies Produced to TDI Isomers

Inhibitor	µg Antigen Conjugate Required for 50% Inhibition of Reaction			
	Anti-2,4 TDI		Anti-2,6 TDI	
	#767	#770	#730	#732
2,4 TDI-GSA	1.0	0.55	>50	>50
2,6 TDI-GSA	>50	>50	0.87	3.6
HDI-GSA	>50	>50	>50	>50
GSA	>50	>50	>50	>50

Table 4

Isomer-Specific Antibody following Absorption of Antisera with Cross-Reactive TDI Isomer

Antiserum	Absorbing Antigen	Test Antigen*	Abs_{410nm}
Anti-2,6 TDI	2,6 TDI-GSA	2,6 TDI-GSA	0.106
"	2,4 TDI-GSA	2,6 TD-GSA	0.208
Anti-2,4 TDI	2,4 TDI-GSA	2,4 TDI-GSA	0.102
"	2,6 TDI-GSA	2,4 TDI-GSA	0.154

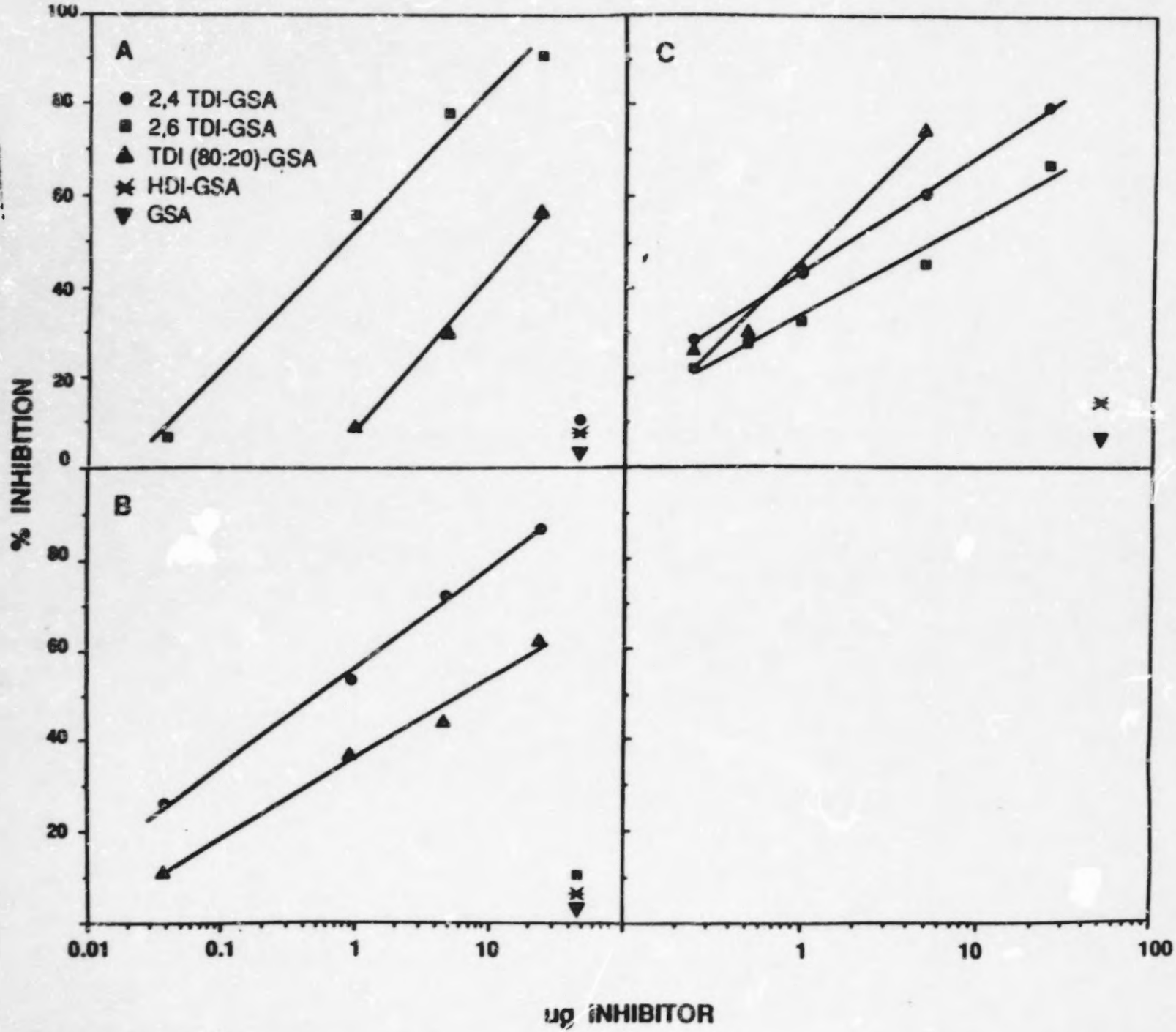
* Antigen used for coating ELISA plate.

Figure 1. Inhibition of binding of anti-TDI antibodies with TDI isomer antigens

A. Anti-2,6 TDI antiserum on 2,6 TDI-GSA plate

B. Anti-2,4 TDI antiserum on 2,4 TDI-GSA plate

C. Anti-TD_{80} antiserum on TD_{80}-GSA plate



CERTIFICATE OF AUTHENTICITY

THIS IS TO CERTIFY that the microimages appearing on this microfiche are accurate and complete reproductions of the records of U.S. Environmental Protection Agency documents as delivered in the regular course of business for microfilming.

Data produced 1 (Month) 29 (Day) 94 (Year) Marcia Tubolino Camera Operator

Place Syracuse (City) New York (State)

AMTEK corp